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**TOXICITY IN THE RAT OF SMOKE PRODUCED BY  
COMBUSTION OF AIRCRAFT AUDIO CABLE INSULATION**

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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<p>The combustion toxicity of three audio cable jacketing materials (polyether polyurethane, thermoset rubber, and thermoset rubber with a flame retardant additive) was investigated by burning known amounts of the insulation materials in a modified FAA exposure system. Douglas Fir was used as a positive control and to provide calibration with other experiments. The FAA exposure system was modified to allow introduction of clean air at the termination of the smoke exposure. Two nonlethal measurements of performance loss in Fisher 344 rats were made using this apparatus. First, the time to unconsciousness was measured as the time from the introduction of smoke to the time at which the animal was unable to walk on the moving surface of a running wheel. Time to recovery was determined as the difference between the time of smoke removal to the time that the animal was able to walk in response to moving the wheel. Results of this study indicate that at low smoke exposures, time to incapacitation is dependent on the material burned, but at higher exposure levels, the material dependency disappeared. Time to recovery is also dependent on the material burned, but this measure was less precise than the time to incapacitation. One of the materials, polyethylene polyether, was observed to be a pulmonary irritant under the exposure conditions used in this experiment.</p>				
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# **TOXICITY IN THE RAT OF SMOKE PRODUCED BY COMBUSTION OF AIRCRAFT AUDIO CABLE INSULATION**

## **INTRODUCTION**

At the request of the Naval Air Warfare Center Aircraft Division Indianapolis (NAWC Indianapolis), the Naval Medical Research Institute (Toxicology) investigated the combustion toxicity of aircraft audio cable wire jacketing material. The objective of this study was to determine the behavioral toxicology of the combustion products from these materials. The insulations investigated were a polyether polyurethane (PEPU), styrene butadiene rubber (SBR), and styrene butadiene rubber with fire retardant (SBRFR).

In the fire situation, loss of capability to perform physical and mental tasks often precedes death, but conventional combustion toxicity evaluations use death as the measured endpoint since performance loss usually results in death. However, during flight aircrew members are called upon to complete complex tasks for survival even in extreme situations, so small performance decrements can significantly affect crew survival. Therefore, this study was conducted using a performance decrement measure as an index of toxicity. Previous studies have investigated the relationship between death and incapacitation. Hidalgo's group modified the lethal endpoint measurement to include an exercise wheel and measured the time until a rat could no longer run as an indicator of incapacitation. They continued exposure to the point where the rat no longer moved as a measurement of time to death (1-4). Similarly, Crane used a motorized wheel as a method to assess loss of ability to walk (5,6). In general, these studies demonstrated that there is a good correlation between time to death and time to incapacitation if the smoke concentration is ultimately lethal (7-9). These observations do not rule out the possibility that there are materials for which the smoke may not be lethal but quite incapacitating.

In the present study, the Crane running wheel apparatus was converted to allow measuring a nonlethal performance endpoint by providing for the admission of fresh air after the exposed animal becomes incapacitated. With this modification, a second measure of smoke toxicity can be made if the time for the exposed animal to regain the ability to walk is measured. Using this approach, both the relative ability of insulation combustion products to produce unconsciousness as well as the duration of unconsciousness can be measured. At the completion of these studies, animals were sacrificed and examined histopathologically to determine if there was any sign of acute organ toxicity. Thus, both the toxic potency and duration of effect produced by the combustion products are estimated in this experiment. These endpoints are relevant to the effects of these combustion products on aircrews in that the time to incapacitation and the length of recovery are critical to aircrew survival in aircraft fire situations.

Since the potential exists for the combustion products from these materials to be respiratory irritants, a second set of experiments were conducted to assess the potential for pulmonary irritancy. These studies provide a measure of cellular injury in the lower respiratory tract and a measure of respiratory tract recovery. These data provide information on the potential for target organ toxicity in addition to the measures of whole animal response represented by the behavioral decrement studies described above.

## **MATERIALS**

Test materials used for these studies were supplied to NMRI/TD by NAWC Indianapolis as samples of production materials. Three classes of material were obtained: polyether polyurethane material (PEPU), thermoset rubber material (SBR), and thermoset rubber material with a flame retardant additive (SBRFR). The materials were supplied either as four-inch square mats or as sprues from casting operations. Douglas fir wood was used as a positive control.

## METHODS

### Inhalation Exposure

Male Fischer 344 rats weighing 240-415 grams ( $\bar{x} = 355$ ) were individually exposed to combustion products from the non-flaming combustion of the test insulation materials. Five repetitions of each exposure were performed. The exposure system used was a modified version of that developed by the Federal Aviation Administration. Modification included the use of a Lindberg tube furnace, insertion of an oxygen monitoring electrode, and sectional glass tubes for breaking the recirculating loop to allow the entry of fresh air. This apparatus is illustrated in Figure 1.

### MODIFIED FAA TYPE EXPOSURE APPARATUS

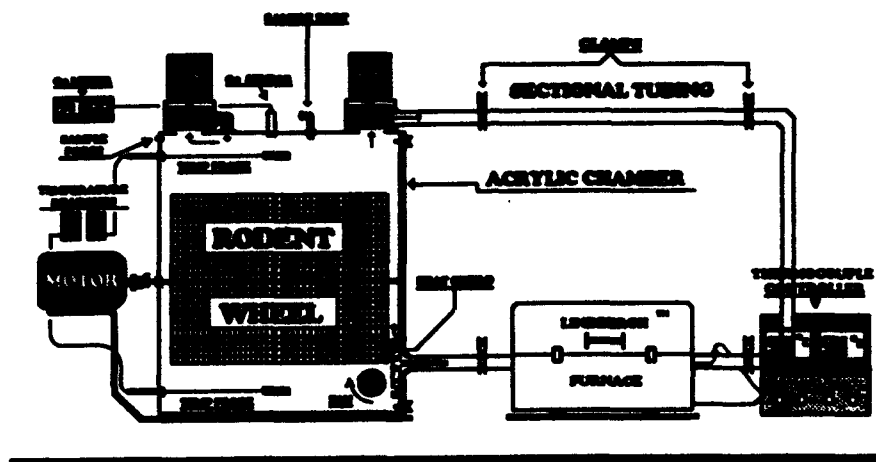


FIGURE 1. Exposure Apparatus for Combustion Toxicity. The exposure system is a modification of the FAA-type exposure apparatus having a total volume of 17 liters.

Prior to the start of the exposure, the test animals were weighed and loaded into the exposure chamber walking wheel. The wheel was then placed into the acrylic chamber and the front cover replaced and tightened. The seams and edges of chamber were then sealed with vacuum grease to prevent combustion product leaks during the combustion phase. The time and monitoring instrumentation values were recorded, and the furnace was engaged. When the optimal furnace temperature set point (500 °C) was reached, a predetermined weight of test material was placed in a ceramic boat and loaded into the center of the furnace. As soon as smoke was observed at the exposure chamber inlet, the recirculation fans were started in order to maintain air circulation, and the walking wheel was engaged. Immediately after the wheel motor was engaged, a timer was activated in order to measure elapsed time to incapacitation.

Immediately after the test animal became incapacitated, the time and monitoring instrumentation values were recorded. Fresh air was then introduced into the system by removing the section of the recirculation loop between the furnace and the exposure chamber and disconnecting the clamp directly to the rear of the furnace tube. In this configuration, the recirculation fans drew fresh air into the exposure chamber, purging the combustion gases. Even though the furnace was shut off three minutes after smoke production was observed, the ceramic boat and any residual test material remained in the furnace and were heated for the duration of the incapacitation phase of the experiment. During the recovery phase of the experiment, gases exhausted from the furnace and exposure chamber were removed from the hood enclosure by a scrubber-equipped hood exhaust

system. Upon completion of each exposure, the weight of the residual test material was recorded.

Preliminary experiments were conducted in order to establish the range of the incapacitation measure employed in this experiment. The comparative testing dose range (CTDR) for this experiment was defined as the dose range empirically determined to produce incapacitation within 30 minutes of exposure but not before 5 minutes after the onset of smoke generation. Incapacitation was defined as the observed inability of an animal to support or right himself with the wheel moving at a linear velocity of approximately 4 cm/sec. For each of the materials tested, the CTDR was determined using an up/down dose procedure with individual test on each of three animals. The initial mass of material burned was approximately one-tenth the reported lethal dose of wood or other rubber compounds. For successive determinations, the dose was doubled until the animal response fell within the defined range. After the CTDR was determined, dose response determinations were carried out on five animals, individually exposed, both in the morning and afternoon for each material tested.

Recovery from the effects of combustion product inhalation was determined by measuring the elapsed time between incapacitation and the time that it took each animal to regain consciousness and walk in response to a momentary rotation of the wheel. After the incapacitation criterion was met, fresh air was immediately introduced into the exposure system, and the wheel was stopped. Every 10 seconds thereafter, the wheel was rotated approximately ninety degrees until the animal was able to stand and walk in response to the wheel's rotation.

#### Chemical Analysis

During exposure and recovery, the percentage of  $O_2$  was monitored using a Hudson oxygen monitor (Model 5590 Hudson Ventronics, Tumecula, CA.). In a separate set of experiments, smoke gases in the inhalation chamber were collected by drawing samples of the chamber atmosphere through a teflon sampling line to an evacuated sample bag at approximately 5 L/min. Chemical analysis of the combustion atmosphere was conducted using a Nicolet 740 fourier transform infrared spectrophotometer (Model 740, Nicolet Instruments, Madison, WI) (FTIR). The instrument was equipped with a 20-m gas cell, an absolute pressure transducer (Baratron 122a, MKS Instruments, Andover, MA), and an MCT-A detector cooled to 77 K with liquid nitrogen. Spectra were acquired with a resolution of  $0.5\text{ cm}^{-1}$  over the spectral range of  $600\text{-}5100\text{ cm}^{-1}$ .

#### Pulmonary Pathology

During the initial experimentation, signs of pulmonary irritation were noted in animals exposed to polyether polyurethane insulation material combustion products. The animals exposed to combustion products from this material were held for 10 days and sacrificed for pathological evaluation of the respiratory tract. Other animals did not exhibit these signs and were not examined histologically.

Previous studies have demonstrated that changes in bronchoalveolar lavage fluid (BALF) LDH and total protein levels are sensitive indicators of lung injury (10-12). Increases in LDH, a cytosolic enzyme, are indicative of cytotoxicity, while changes in total protein suggest exudation of plasma proteins, *i.e.* alterations in the alveolar-capillary barrier (12-13). Therefore, an additional experiment was carried out to assess the pulmonary irritation potential of polyether polyurethane insulation material combustion products. Groups of three rats were individually exposed to the combustion products of 0.4 g of Douglas Fir and 0.4 g of polyether polyurethane. Exposure continued until the each rat was incapacitated. Three naive animals served as a control group. Immediately after exposure, two rats were euthanized with 50 mg/kg of pentobarbital. Single animals were held one week after exposure to determine the extent of recovery. The abdomen was opened and the trachea cannulated. The lung was lavaged three times with 10 ml of phosphate buffer solution (PBS) and the washings combined and centrifuged (500xg for 10 min). The supernatant (BALF) was analyzed for total protein and lactate dehydrogenase (LDH) activity. An increase in total protein was taken as indicative of an

inflammatory response suggestive of an alteration in alveolar capillaries. Increased LDH was taken as a marker of cytotoxicity (cellular injury).

### Histopathology

Test and control animals were euthanized with carbon dioxide vapor. Complete necropsies were performed, and gross findings were recorded during prosection. In addition to tissues with gross lesions, the tissues collected for histopathologic examination were: heart, larynx, lung, trachea, nasal turbinates, salivary gland, mandibular lymph node, esophagus, spleen, liver, kidney, urinary bladder and vesicular gland. Tissues collected for histopathologic examination were fixed in 10% neutral-buffered formalin and trimmed for histologic processing. Via routine methods (14), the tissues were embedded in paraffin, sectioned at 4 to 7  $\mu\text{m}$ , and stained with hematoxylin and eosin.

## **RESULTS**

### Inhalation Exposure

Combustion of the materials tested was primarily by pyrolysis and charring; flaming conditions were not observed. Table I shows the weight range of material burned for each material and the lowest oxygen concentration observed during the experiment.

**TABLE I. Percentage Oxygen Concentration Found During Pyrolysis of Test Materials as a Function of Amount of Material Combusted**

<u>Material</u>	<u>Amount Combusted (mg)</u>	<u>%O<sub>2</sub> at Incapacitation</u>
Douglas Fir	200	N/A
Douglas Fir	400	18 $\pm$ 0.01
Douglas Fir	800	17 $\pm$ 0.71
Douglas Fir	1600	16.4 $\pm$ 0.55
SBR	200	18.2 $\pm$ 0.45
SBR	400	17.4 $\pm$ 0.55
SBR	800	17.0 $\pm$ 0.71
SBR	1600	15.6 $\pm$ 0.89
SBRFR	200	18.25 $\pm$ 0.5
SBRFR	400	17.2 $\pm$ 0.44
SBRFR	800	16 $\pm$ 0.01
SBRFR	1600	13.6 $\pm$ 0.55
PEPU	200	N/A
PEPU	400	18.25 $\pm$ 0.5
PEPU	800	17.4 $\pm$ 0.89
PEPU	1600	15.6 $\pm$ 1.14



Tables II and III show the mean and standard error of the incapacitation and recovery times for each material. These exposures were conducted with relatively low amounts of test materials; lethal concentrations of smoke were not reached, and no animals died of exposure related effects; however, animals exposed to higher levels of polyether polyurethane combustion products exhibited nasal exudates. Euthanasia and pathological examination of the animals displaying these symptoms ten days after exposure indicated no residual tissue damage from inhalation of the polyether polyurethane insulation combustion products.

**TABLE II. Mean ( $\pm$  S.E.M.) Exposure time(s) to Incapacitation**

	<u>200 mg</u>	<u>400 mg</u>	<u>800 mg</u>	<u>1600 mg</u>
PEPU	1800.00 (0.0)	904.82 (42.5)	707.44 (117.1)	373.23 (35.3)
SBR	875.62 (57.1)	714.96 (176.6)	590.83 (120.1)	351.38 (17.9)
SBRFR	772.91 (43.8)	561.93 (97.0)	464.42 (23.7)	317.24 (21.3)
DOUGAS FIR	1686.00 (74.9)	756.22 (55.1)	443.60 (22.8)	345.00 (14.2)

**TABLE III. Mean ( $\pm$  S.E.M.) Duration (s) from Incapacitation to Recovery of Function**

	<u>200 mg</u>	<u>400 mg</u>	<u>800 mg</u>	<u>1600 mg</u>
PEPU	0.00 (0.0)	95.24 (10.7)	84.79 (8.0)	86.39 (7.2)
SBR	59.23 (11.2)	55.19 (8.5)	54.23 (5.8)	75.22 (10.7)
SBRFR	122.19 (36.2)	97.37 (8.5)	113.03 (23.4)	129.7 (18.8)
DOUGLAS FIR	27.00 (17.0)	37.62 (4.5)	70.82 (12.6)	132.0 (22.4)

### Incapacitation

When the results of the four doses of materials tested which lead to incapacitation were analyzed, reliable differences in time to incapacitation were found to be produced by: Material Type [ $F(3,64) = 24.96$ ,  $p < .0001$ ]; Dose [ $F(3,64) = 118.00$ ,  $p < .0001$ ]; and the interaction between Material Type and Dose [ $F(9,64) = 11.18$ ,  $p < .0001$ ]. The plotted data (Figure 2) suggested that much of the Material effect and most of the Interaction effect was attributable to differences in incapacitation at the lowest dose (200 mg) where the effects of Douglas fir and Polyether polyurethane appeared to be substantially less incapacitating than those of the two butyl rubber compounds. In order to test this hypothesis, an ANOVA was recalculated using only the 400, 800, and 1600 mg data. While this analysis yielded a reliable effect of Dose [ $F(2,48) = 23.45$ ,  $p < .0001$ ], the reliability effect attributable to Material Type was substantially attenuated [ $F(3,48) = 3.79$ ,  $p > .015$ ] and the effect attributable to the interaction of Dose with Material Type was obliterated [ $F(6,48) = 0.89$ ,  $P > .500$ ].

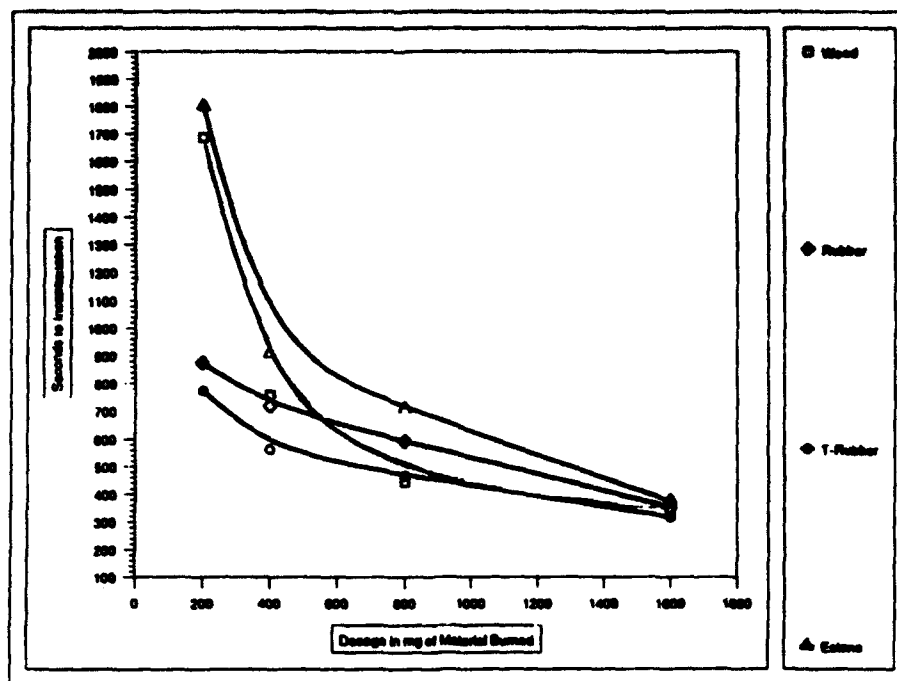


FIGURE 2. Time to Incapacitation Versus Amount of Wire Insulation Material Burned. Time is measured in seconds from the start of smoke exposure.

### Recovery

When the results of incapacitating doses of materials were analyzed (Figure 3), reliable differences in time to recovery were found to be produced by: Material Type [ $F(3,63) = 8.35, p < .0001$ ]; Dose [ $F(3, 63) = 10.92, p < .0001$ ]; and also by the Interaction between Material Type and Dose [ $F(9,63) = 3.50, p < .0015$ ].

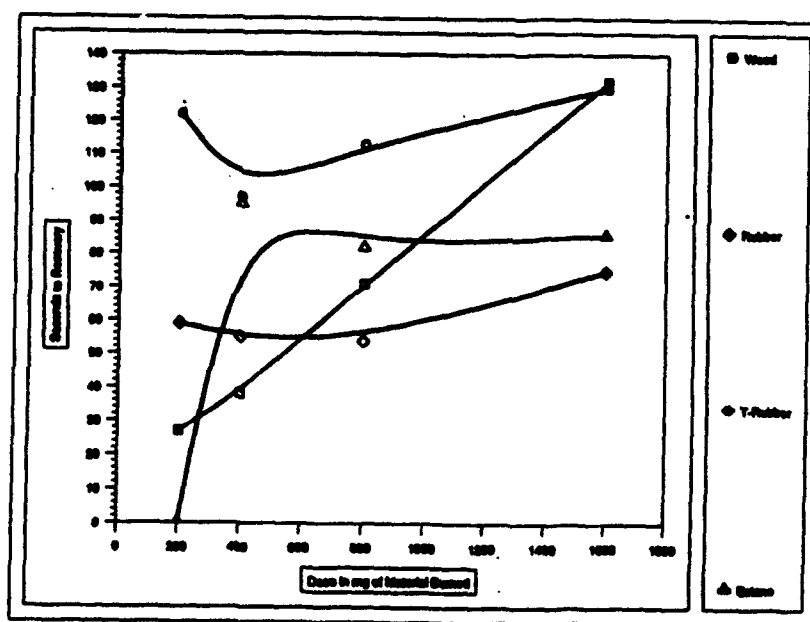
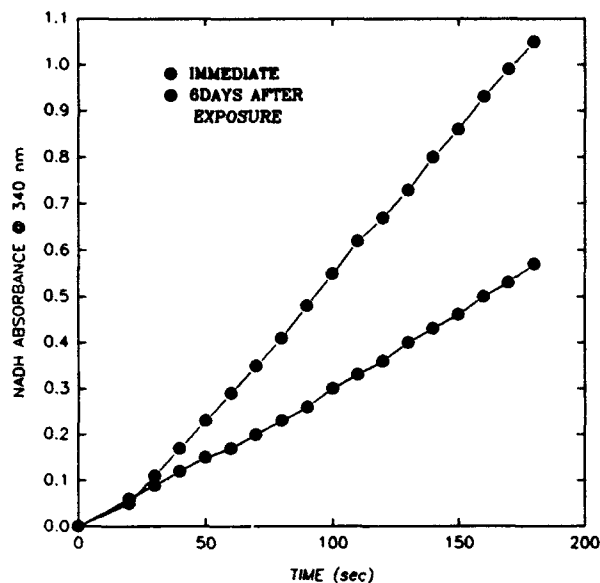


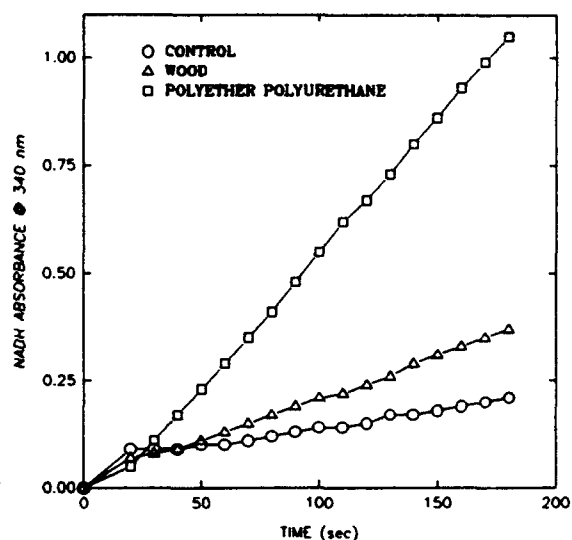
FIGURE 3. Time to Recovery of Consciousness Versus Amount of Wire Insulation Material Burned. Time to recovery is measured from the onset of unconsciousness to the time when the rat regains the ability to walk.

## Pulmonary Irritation

Bronchoalveolar Lavage Fluid (BALF) Lactate Dehydrogenase (LDH) assay data are shown in Figures 4 and 5. As seen in Figure 4, LDH activity in the BALF from rats immediately after exposure to smoke decreases in the order PEP < wood < control. Figure 5 illustrates that the LDH in BALF immediately after exposure to PEP smoke is higher than that observed in animals that are allowed to recover for six days.



**Figure 4** Lactate Dehydrogenase Activity Measured as Increasing Absorbance at 340 nm. Animals were lavaged immediately after exposure to polyether polyethylene smoke or after recovery for 6 days.



**Figure 5** Lactate Dehydrogenase Activity in BALF. LDH activity as measured by increasing NADH absorbance as a function of time.

The results for total BALF protein determinations are shown in Table IV. These measurements indicate that BALF protein is elevated after exposure to the combustion products of the materials studied.

**TABLE IV. Total Bronchoalveolar Lavage Fluid Protein**

<u>EXPOSURE</u>	<u>TOTAL BALF PROTEIN <math>\mu\text{g/ml}</math></u>
CONTROL	56.3
WOOD	142.5
POLYETHYLENE POLYETHER (IMMEDIATE)	237.5
POLYETHYLENE POLYETHER (6 DAY DELAY)	110.0

## DISCUSSION

### Incapacitation

These results suggest that there is a substantial difference between the capacities of the burned materials to produce total incapacitation, but only at the lowest doses. At low doses, polyether polyurethane combustion produced no incapacitation, and Douglas fir combustion produced minimal incapacitation, while combustion products from both butyl-rubber compounds rapidly incapacitated the animals. As the amount of material combusted was increased, these differences were significantly reduced. At the highest dose tested (1600 mg), an amount of material which produced almost complete smoke-obscuration of the animals in the chamber, differences in incapacitation time among the materials tested were not statistically significant. For all doses tested, polyether polyurethane exposure resulted in numerically longer times to incapacitation than either of the butyl-rubber compounds. At doses 400 mg or greater, however, polyether polyurethane produced marked respiratory irritancy, evidenced by excessive nasal discharge not observed after exposure to either of butyl-rubbers or Douglas fir combustion products.

### Recovery

The results from the recovery tests are somewhat less conclusive than those from the tests of incapacitation, due to higher degrees of within-group variation. While it is relatively easy to operationally define the conditions under which an animal can be classified as being incapacitated, it is much harder to define those conditions which mark the return of consciousness and the reestablishment of behavioral capacity in the animal. Even though the measurement of recovery from incapacitation proved to be more variable than the incapacitation measure itself, the results obtained augment those from the incapacitation measure. Since no animals exposed to polyether polyurethane smoke were incapacitated at the lowest dose (200 mg), recovery time for this group of animals was zero. For higher doses, recovery time from polyether polyurethane-smoke induced incapacitation was between the two butyl-rubber compounds. Additionally, in marked contrast to the results from the incapacitation measures, smoke from the fire retarded butyl-rubber compound was more toxic than the non-treated butyl-rubber formulation as measured by time to recover after incapacitation.

### Pulmonary Irritation

The data in Figures 4 and 5 as well as that contained in Table IV allow analysis of the effects of the combustion products on lung tissue. The LDH specific activities calculated from this data are shown in Table V.

**Table V. Bronchoalveolar Lavage LDH Specific Activity After Exposure to Combustion Products.**

<u>EXPOSURE</u>	<u>LDH SPECIFIC ACTIVITY</u> <u>nmoles/mg/min.</u>
CONTROL	26.6
WOOD	33.7
POLYETHYLENE POLYETHER (IMMEDIATE)	73.3
POLYETHYLENE POLYETHER (6 DAY DELAY)	73.6

Overall BALF protein (Table IV) is elevated after exposure when compared to controls, indicating that there is cell damage or protein leakage into the air space. Similarly, LDH specific activity is elevated after

## APPENDIX A

As described in the chemical analysis section in the main body of this report, smoke gases in the inhalation chamber were collected by drawing samples of the chamber atmosphere through a teflon sampling line to an evacuated sample bag at approximately 5 L/min. Chemical analysis of the combustion atmosphere was conducted using a Nicolet 740 fourier transform infrared spectrophotometer (Model 740, Nicolet Instruments, Madison, WI)(FTIR). The instrument was equipped with a 20-m gas cell, an absolute pressure transducer (Baratron 122a, MKS Instruments, Andover, MA), and an MCT-A detector cooled to 77 K with liquid nitrogen. Spectra were acquired with a resolution of  $0.5\text{ cm}^{-1}$  over the spectral range of  $600\text{--}5100\text{ cm}^{-1}$ .

Collected samples were transferred to the FTIR gas cell for analysis by exposing the sample bag combustion atmosphere to the gas cell at an initial pressure of 4mm Hg. Sample transfer into the 20-meter gas cell was assumed to have been completed when the pressure inside the cell reached the nominal laboratory atmospheric value of 743 mm Hg. Sample analysis was performed with the aid of the Nicolet automated data analysis programs and the corresponding reference data files. The gases analyzed were  $\text{CO}_2$ ,  $\text{CO}$ ,  $\text{COS}$ ,  $\text{COCl}_2$ ,  $\text{HCN}$ ,  $\text{HCl}$ ,  $\text{HF}$ ,  $\text{NH}_3$ ,  $\text{H}_2\text{S}$ ,  $\text{H}_2\text{O}$ ,  $\text{SO}_2$ ,  $\text{NO}_2$ ,  $\text{H}_2\text{CO}$  (formaldehyde) and acrolein. In addition,  $\text{CH}_4$  (methane) and  $\text{C}_2\text{H}_4$  (ethylene) were identified but not quantitated.

Figures A1a, A1b, A1c and A1d are the IR spectra obtained from the combustion gases of wood, SBR, SBRFR, and polyether polyurethane respectively. Qualitatively, these spectra are very similar in terms of the gases produced during pyrolysis. Under the conditions of the combustion studies,  $\text{CO}_2$ ,  $\text{CO}$ ,  $\text{HCN}$ ,  $\text{H}_2\text{O}$ ,  $\text{SO}_2$ , and  $\text{H}_2\text{CO}$  were detected. Major absorption bands were invariably due to  $\text{CO}_2$  and  $\text{CO}$ .  $\text{CO}_2$  signatures were found at  $718$  and  $2300\text{ cm}^{-1}$ . High energy, but weakly absorbing bands were also observed for this gas at  $4978$

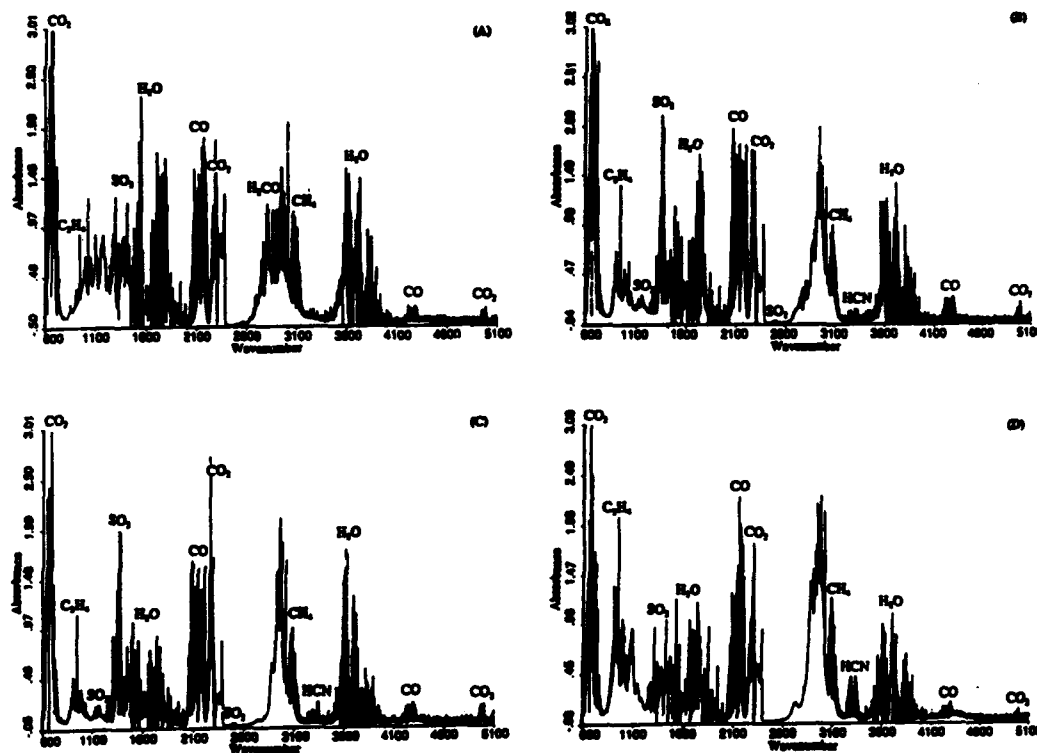


Figure A1 Infrared Spectra Obtained From Combustion of Wire Insulation Materials and Wood.  
a). wood b). SBR c). SBRFR and d). polyether polyurethane.

## REFERENCES

1. Hidalgo, C. J., and L.A. Gall. 1977. Relative toxicity of pyrolysis products of some wood samples. J. Combust. Toxicol. 4: 193-199.
2. Hilado, C. J., Huttlinger, N. V. and B. A. O'Neill. 1978. Effect of heating rate on toxicity of pyrolysis gases from some wood samples. J. Combust. Toxicol. 5: 25-38.
3. Hilado, C. J., Marcussen, W. H., Furst, A and H.A. Leon. 1976. Effect of species on relative toxicity of pyrolysis gases. J. Combust. Toxicol. 3: 125-134.
4. Hilado, C. J. and J.E. Schneider. 1979. Toxicity of pyrolysis gases from polytetrafluorethylene. J. Combust. Toxicol. 6: 91-98.
5. Hilado, C. J., and C.R. Crane. 1977. Comparison of results with the USAF/NASA and FAA/CAMI toxicity screening test methods. J. Combust. Toxicol. 4: 56-60.
6. Hilado, C. J., Cumming, H. J., Machado, A. M., Schneider, J. E., Crane, C. R., Sanders, D. C., Endecott, B. R. and J.K. Abbott. 1977. Comparison of animal responses to the combustion products generated by two test procedures, the USAF/NASA methodology and the FAA/CAMI system. J. Combust. Toxicol. 4: 325-359.
7. Gad, S. C. and A.C. Smith. 1983. Influence of heating rates on the toxicity of evolved combustion products: results and a system for research. J. Fire Sci. 1: 465-479.
8. Hartung, R., Ball, G. L., Boettner, E. A., Rosenbaum, R. and Z.R. Hollingsworth. 1977. The performance of rats on a rotorod during exposure to combustion products of rigid polyurethane foams and wood. J. Combust. Toxicol. 4: 506-522.
9. Mitchess, D. S., Rogers, W. R., Herrera, W. R and W.G. Switzer. 1978. Behavioral incapacitation of rats during full-scale combustion of natural-fiber and synthetic polymeric furnishings. Fire Res. 1: 187-197.
10. Sykes, S. E., Morgan, A., Evans, J. C., Evans, N., Holmes, A., and R.A. Moores. 1982. Use of an *in-vivo* test system to investigate the acute and sub-acute responses of the rat lung to mineral dusts. Ann. Occup. Hyg. 26: 593-605.
11. Beck, B. D., Brain, J. D., and D.E. Boheannon. 1982. An *in-vivo* hamster bioassay to assess the toxicity of particulates for the lungs. Toxicol. Appl. Pharmacol. 66: 9-29.
12. Henderson, R. F. 1984. Use of bronchoalveolar lavage to detect lung damage. Environ. Health Perspect. 56:115-129.
13. Henderson, R. F., Benson, J. M., Hahn, F. F., Hobbs, C. H., Jones, R. K. Mauderly, J. L., McClellan, R. O. and J.A. Pickrell. 1985. New approaches for the evaluation of pulmonary toxicity: Bronchoalveolar lavage fluid analysis. Fundam. Appl. Toxicol. 5: 451-458.
14. Luna, L. G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed. 258 pp. McGraw-Hill, New York.

cm<sup>-1</sup>. The strongly absorbing P and Q branches characteristic of CO were centered on the 2150 cm<sup>-1</sup> region and its overtone at 4300 cm<sup>-1</sup>. Wide bands in the regions 3200-4100 and 1200-2000 cm<sup>-1</sup> are due to water. The three polymeric materials pyrolyzed in this study produced HCN as evidenced by the presence of its P and Q branches centered on the 3275 cm<sup>-1</sup> region. All three materials also produced the irritant SO<sub>2</sub> following their pyrolysis. The SO<sub>2</sub> signature is unique in that it resembles a typical first derivative peak. The peak inflection point was located at 1363 cm<sup>-1</sup>. Quantitation by FTIR leads to the data shown in Table AI.

**TABLE AI. Concentration of Gases Produced During the Pyrolysis of Douglas Fir, SBR, SBRFR and PEPU**

<u>GAS</u>	<u>DOUGLAS FIR</u>	<u>SBR</u>	<u>SBRFR</u>	<u>PEPU</u>
CO <sub>2</sub>	1275 ppm	1519 ppm	1397 ppm	901 ppm
CO	234 ppm	226 ppm	205 ppm	219 ppm
COS	N.D.	N.D.	N.D.	N.D.
COCl <sub>2</sub>	N.D.	N.D.	N.D.	N.D.
HCN	N.D.	7 ppm	16 ppm	20 ppm
HCl	N.D.	N.D.	N.D.	N.D.
HF	N.D.	N.D.	N.D.	N.D.
NH <sub>3</sub>	N.D.	N.D.	N.D.	N.D.
H <sub>2</sub> S	N.D.	N.D.	N.D.	N.D.
H <sub>2</sub> O	0.13%	0.10%	0.10%	0.07%
SO <sub>2</sub>	6 ppm	76 ppm	82 ppm	5 ppm
NO <sub>2</sub>	N.D.	N.D.	N.D.	N.D.
H <sub>2</sub> CO	72 ppm	N.D.	N.D.	N.D.
C <sub>3</sub> OH <sub>4</sub>	N.D.	N.D.	N.D.	N.D.

\*Abbreviations: SBR (styrene butadiene); SBRFR (styrene butadiene with fire retardant); PEPU (polyetherpolyurethane); N.D. (not detectable).

Data obtained from these chemical analyses must be regarded as preliminary because the methodology being used is under development, and they were collected from combustion experiments done in the absence of animals, not in the exposure studies described in the body of the report. However, they offer insight into the differences in the combustion products of these three materials. The data in Table III indicate that the four materials burned produced roughly equivalent amounts of CO<sub>2</sub> and CO; however, both wood and the SBR rubber produced low levels of HCN while the polyether polyurethane and fire retardant (SBRFR) rubber produced nearly equal HCN levels. SBRFR rubber produced the highest level of SO<sub>2</sub> and significant HCN. The polyether polyethylene produced an intermediate level of HCN and no SO<sub>2</sub>. SBR rubber produced low HCN levels and SO<sub>2</sub> levels similar to SBRFR. Additional method development and animal studies are necessary to determine the relationships between these findings and those of the animal bioassays.

exposure, indicating that cell damage has occurred. The greater pneumotoxic potential of polyether polyurethane combustion products relative to wood was clearly reflected in the degree of changes in BALF total protein and LDH. Polyether polyurethane combustion products caused a two-fold increase in BALF markers of lung injury compared to wood at the same dose level. After six days of recovery, the BALF analysis showed a 50% decrease in total protein with no change in LDH specific activity. These data suggest recovery from the alveolar-capillary damage, but no recovery from the exposure-induced cytotoxicity.

### General

Using incapacitation as a measure of toxic effect, the dose response characteristics of the materials tested indicate a difference among the materials at the lowest smoke concentrations used. At these smoke concentrations, the wood and polyether polyurethane insulation smokes were significantly less incapacitating than were the thermosetting rubber combustion products. As the smoke concentration increased to intermediate values, these differences disappeared. As the amount of material burned was increased, the time to incapacitation decreased to a nearly equivalent value for all materials tested. It should be noted that as the amount of material burned increased, the levels of oxygen available to the animal decreased, and the levels of CO<sub>2</sub> increased. This suggests that in the early stages of an actual fire situation, both the polyether polyurethane insulation and wood combustion provide an equivalent impediment to continued function while breathing the smoke. Burning thermoset rubber insulation would produce a smoke that would impede normal function more quickly. By the time the burning area becomes densely smoke-filled, these data suggest that there would be no difference in incapacitating effect among smokes from the materials tested.

The results obtained from the recovery measures are consistent with the results obtained from the incapacitation measures. Recovery time from the polyether polyurethane smoke was between that obtained for smoke from the two thermosetting rubber materials. The fire retardant treated thermosetting rubber had consistently longer recovery times than did the identical material without the fire retardant, implying that the addition of the fire retardant increased the toxicity of the material when recovery is used as a measure of toxicity. The polyether polyurethane smoke exhibited a threshold for effects that reflects the fact that incapacitation did not occur at the lowest dose. Above the 200 mg dose group, the recovery time for each of the three materials was independent of the material or the amount burned.

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